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FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
MBHB00-1260	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	
09/719437	
INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE
PCT/FR99/01409	14 June 1999
PRIORITY DATE CLAIMED	
12 June 1998	

TITLE OF INVENTION
HIV Virus Mimotopes

APPLICANT(S) FOR DO/EO/US
Veronique Barban

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1)
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau)
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5))

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Acknowledgement Postcard
Patent Data Sheet

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/719437	PCT/FR99/01409	MBHB00-1260

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $690.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	34 - 20 =	14	x $18.00	**$252.00**
Independent claims	1 - 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☒	**$270.00**
TOTAL OF ABOVE CALCULATIONS			=	**$1,382.00**
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	**$0.00**
SUBTOTAL			=	**$1,382.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**
TOTAL NATIONAL FEE			=	**$1,382.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**
TOTAL FEES ENCLOSED			=	**$1,382.00**
			Amount to be: refunded	$
			charged	$

☒ A check in the amount of **$1,382.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **13-2490** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

MCDONNELL BOEHNEN HULBERT & BERGHOFF
300 South Wacker Drive
Suite 3200
Chicago, Illinois 60606

SIGNATURE

Kevin E. Noonan
NAME

35,303
REGISTRATION NUMBER

December 11, 2000
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :
U.S. National Serial No. :
Filed :
PCT International Application No. : PCT/FR99/01409

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below;

That I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the international application No. PCT/FR99/01409 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: 24 November 2000

Full name of the translator : Abraham SMITH
For and on behalf of RWS Group plc

Post Office Address : Europa House, Marsham Way,
Gerrards Cross, Buckinghamshire,
England.

Express Mail Label: EL602856962US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Attorney Docket No. 00-1260)

In the Application of:	)	
	)	
V. Barban	)	
	)	**Examiner: TBA**
Serial No.: US Nat'l Phase of PCT/FR99/01409	)	
	)	**Group Art Unit: 1807**
Filing Date: Int'l Filing Date June 14, 1999	)	
	)	
For: HIV Virus Mimotopes	)	

PRELIMINARY AMENDMENT

Asst. Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please consider the following amendments and remarks made in response to the Office Action mailed

AMENDMENTS

In the claims:

Please cancel claims 16-19.

Please amend the remaining claims as follows:

1. (Amended) [Peptide] An anti-HIV peptide for the prevention or therapeutic treatment of HIV virus infection [capable of interacting] that interacts with an antibody specific for an antigen of the envelope of said virus and obtained from an HIV-positive patient belonging to the "long-term nonprogressor" group, comprising an amino acid sequence which mimics a conformational epitope of an antigen of said envelope [without, however,] wherein the amino acids sequence does not correspond[ing] to a continuous amino acid sequence of this antigen.

2. (Amended) [Peptide] The anti-HIV peptide according to claim 1, [according to which] wherein the antigen [of the envelope] is [represented by] the envelope protein gp160.

3. (Amended) [Peptide] The anti-HIV peptide according to claim 1 [or 2], [characterized in that this] wherein the peptide [may comprise the sequences 1 to 11] is selected from the group consisting of:

SEQ. ID. NO.: 1 Phe Asn Leu Thr His Phe Leu,

SEQ. ID. NO.: 2 Glu Gly Trp His Ala His Thr,

SEQ. ID. NO.: 3 Lys Leu Asn Trp Met Phe Thr,

SEQ. ID. NO.: 4 Ser Thr Asn Trp Met Phe Thr,

SEQ. ID. NO.: 5 Ala Met Pro Leu Pro Tyr Thr Phe,

SEQ. ID. NO.: 6 Asp Ser His Thr Pro Gln Arg,

SEQ. ID. NO.: 7 Val Ser Phe Thr Pro Ser Phe

SEQ. ID. NO.: 8 His Ala Ala Leu Ser Met Asn Thr His Ala Leu Met,

SEQ. ID. NO.: 9 Ala Trp His Glu Ser Arg Ala,

SEQ. ID. NO.: 10 Phe Lys Thr Ala Tyr Pro Thr, and

SEQ. ID. NO.: 11 Ser His Ala Leu Pro Leu Thr Trp Ser Thr Ala Ala.

4. (Amended) [Peptide] A peptide comprising [the linkage of] at least two anti-HIV peptides according to [one of claims 1 to] claim 3.

5. (Amended) [Peptide] The peptide according to claim 4, [comprising the duplication of identical] wherein the anti-HIV peptides are identical.

6. (Amended) [Peptide] The peptide according to claim 5, [in which the coupling of] wherein the two anti-HIV peptides [occurs] are linked by means of a ["spacer"] spacer arm consisting of the amino acid sequence Gly Pro Gly.

7. (Amended) [Conjugate] A conjugate comprising at least one peptide according to [one of claims 1 to 6,] claim 3 bound to a carrier molecule [in order to induce or enhance the immunogenicity of said peptides], wherein the immunogenicity of the peptide is induced or enhanced.

8. (Amended) [Conjugate] The conjugate according to claim 7, [according to which] wherein the carrier molecule comprises at least one helper T epitope of the HIV virus.

9. (Amended) [Conjugate] The conjugate according to claim 8, [whose] wherein the carrier molecule comprises [the] HIV p24E [and] or T1 epitopes [of the HIV virus].

10. (Amended) [Conjugate] The conjugate according to claim 9, comprising a first peptide [resulting from the duplication of] selected from the sequences NO: 1, NO: 3 [or] and NO: 4 [according to claim 6] linked to an identical second peptide by a Gly Pro Gly spacer to form a linked peptide, said linked peptide being bound on the N-terminal side by a spacer to the p24E epitope and the C-terminal side by a spacer to the T1 epitope [by means of two "spacer" arms].

11. (Amended) [Conjugate] The conjugate according to claim 10, [characterized in that] wherein the [2 spacer arms] spacers bound to the p24E and T1 epitopes are identical and consist of the linkage Gly Pro Gly and [in that it] the linked peptide comprises any one of [the sequences] SEQ. ID. NOs.: 12 to 14:

SEQ. ID. NO.: 12 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Lys Leu Asn Trp Met Phe Thr Gly Pro Gly Lys Leu Asn Trp Met Phe Thr Gly Pro Gly Lys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

SEQ. ID. NO.: 13 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Ser Thr Asn Trp Met Phe Thr Gly Pro Gly Ser Thr Asn Trp Met Phe Thr Gly Pro Gly Lys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

SEQ. ID. NO.: 14 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Phe Asn Leu Thr His Phe Leu Gly Pro Gly Phe Ans Leu Thr His Phe Leu Gly Pro Gly Lys Gln Ile Lie Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

12. (Amended) [Recombinant] A recombinant vector comprising a functional expression cassette [allowing the expression of] comprising a polynucleotide encoding a peptide according to one of claims [1 to 11] 3, 4, 8, and 11.

13. (Amended) [Recombinant] The recombinant vector according to claim 12, [characterized in that it] wherein the vector is an adenovirus, a poxvirus, a baculovirus, a bacteriophage or a plasmid.

14. (Amended) [Therapeutic] A therapeutic or prophylactic composition for HIV infection[, in particular intended for vaccine use, whose] wherein the active ingredient comprises a peptide according to one of claims [1 to 11] 3, 4, 8, and 11 and/or a recombinant vector encoding said peptide [according to either of claims 12 and 13].

15. (Amended) [Composition] The composition according to claim 14, [whose] wherein the active ingredient is [in the form of a formulation] combined with a compatible adjuvant for administration of an effective dose by the mucosal or parenteral route.

20. A method of diagnosing HIV comprising measuring the humoral and/or cell-mediated immune response of a blood sample contacted with a peptide according to [one of] claims 3 or 4.

21. A method of diagnosing HIV comprising measuring the humoral and/or cell-mediated immune response of a blood sample contacted with a conjugate according to claims 8 or 11.

22. A method of preventing or treating HIV comprising stimulating immune system cells of a patient *in vitro* with a peptide according to claims 3 or 4 and then re-injecting the cells into the patient's body.

23. A method of preventing or treating HIV comprising stimulating immune system cells of a patient *in vitro* with a conjugate according to claims 8 or 11 and then re-injecting the cells into the patient's body.

REMARKS

The amendments made herein are solely for purposes of bringing the claims into conformance with standard U.S. practice and to reduce the costs of filing. No new subject matter has been added by these amendments.

If there are any questions or comments regarding this preliminary amendment or application, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully submitted,

Date: December 11, 2000

Kevin E. Noonan

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Mimotopes of the HIV virus

Subject of the invention

The present invention relates to the treatment and prevention of the HIV virus and in particular to any peptide mimicking novel conformational epitopes of antigens of the HIV virus envelope and to any polynucleotide integrated into a vector allowing the expression of said peptides and their use for therapeutic, prophylactic, in particular vaccine, and/or diagnostic purposes.

Field of the invention

HIV is an enveloped RNA virus and represents the etiological agent of the acquired immunodeficiency syndrome or AIDS, whose outcome is ultimately fatal, characterized by a progressive destruction of the immune system and the concomitant development of microbiological infections often involving opportunistic microorganisms.

The majority of individuals develop an acquired immunodeficiency syndrome within 10 years following infection. Indeed, the immune reactions in response to the infection are very often inadequate and in particular those which are directed against the envelope proteins precisely because of the very high variability of the virus resulting from its high capacity to multiply, to mutate and to recombine (Bangham C.R.M. et al 1997 Lancet 350: 1617-1621). The very high variability of the HIV virus makes it possible to escape control by the immune system and thus promotes dissemination of the virus. However, recent epidemiological studies have shown that some infected persons could contain their infection, with no apparent clinical manifestations and with no biological signs of immunosuppression for periods of longer than 10 years (Pilgrim A.K. et al., 1997, J. Infect. Dis. 176: 924-932). The serum of these persons, also called "long-term nonprogressors", reveals the presence of

neutralizing antibodies against primary isolates of the HIV virus (Pilgrim A.K. et al., 1997, J. Infect. Dis. 176: 924-932).

The HIV virus envelope, derived from the product of expression of the Env gene (envelope gene), is synthesized first of all in the form of a glycoprotein Gp160 which is then cleaved into two glycoproteins Gp 120 and Gp 41. These 3 proteins are found at the surface of cells infected with HIV. Furthermore, Gp 160 and Gp 120 possess affinity for the CD4 molecule present at the surface of certain T lymphocytes, a CD4 molecule which serves as port of entry for the HIV virus toward the inside of the cell. Up until now, very few epitopes accessible to the immune system have been described on the HIV virus envelope (Burton D.R, 1997 Proc. Natl. Acad. Sci. USA 94: 10018-10023).

A need therefore exists for the characterization of novel epitopes of the HIV envelope.

A need also exists to identify epitopes of the envelope which are inducers of neutralizing antibodies, or in other words, which are capable of reducing or suppressing viral dissemination.

A need also exists to identify a pharmaceutical composition which makes it possible to effectively treat or to prevent HIV virus infection.

Finally, a need also exists to develop reagents entering in particular into the composition of immunological kits which make it possible to distinguish in particular among infected persons those which are most resistant to infection or "long-term nonprogressors" and to test, for example, the efficacy of novel vaccines by their capacity to induce neutralizing antibodies.

Summary of the invention

The present invention aims to satisfy these needs by identifying novel peptide structures for the therapeutic or prophylactic treatment of HIV virus infection from the use of a combinatorial antibody

library obtained from HIV-positive patients belonging to the "long-term nonprogressor" group. To this effect, the invention relates to any peptide structure capable of reacting with an antibody specific for an antigen of the HIV envelope derived, for example, from a combinatorial antibody library obtained from lymphocytes of HIV-positive patients belonging to the "long-term nonprogressor" group, comprising an amino acid sequence which mimics a conformational epitope of the envelope of said virus without, however, corresponding to a continuous amino acid sequence of this antigen.

The selection of mimotopes by means of recombinant antibodies obtained from HIV-positive patients belonging to the "long-term nonprogressor" group has the advantage of identifying novel epitopes inducing neutralizing antibodies which are effective in protecting against primary HIV virus isolates.

The present invention also relates to any recombinant vector comprising a functional expression cassette allowing the expression of a polynucleotide encoding a peptide meeting the criteria defined above.

The present invention also relates to a therapeutic or prophylactic composition for the HIV virus, in particular intended for vaccine use, whose active ingredient comprises a peptide meeting the criteria defined above and/or a recombinant vector encoding said peptide.

Finally, the present invention also relates to

- the use of a peptide meeting the criteria defined above as reagent for the diagnosis of the HIV virus which makes it possible in particular to identify subjects in contact with the virus who are more resistant to infection, said diagnosis comprising the evaluation, from a blood sample, of the humoral and/or cell-mediated response specific for this peptide;
- the use of a peptide meeting the criteria defined above and/or of a recombinant vector encoding said peptide for the preparation of a therapeutic or

prophylactic composition intended for the treatment or prevention of HIV virus infection.

Description of the invention

In the context of the present invention, various terms used are defined below:

The expression "peptide" is understood to mean a sequence of at least 6 amino acids linked to each other by a peptide bond, obtained by chemical synthesis or by genetic recombination techniques, preferably between 6 and 100 amino acids and in particular between 20 and 80 amino acids.

The expression "antigen of the HIV envelope" is understood to mean any molecular entity which binds to an antibody specific for any product derived from the env gene, comprising in particular gp 160 in natural or recombinant form, its derivatives consisting of gp41 and gp 120 which may also be in natural or recombinant form and the products derived from the combination of said molecules.

The expression "conformational epitope" is understood to mean a three-dimensional structure which allows its positioning in the specific binding site of an antibody, in the manner of a key in a lock, and which is represented by an amino acid sequence which does not correspond to a continuous amino acid sequence of the protein against which this antibody is directed. Preferably, this amino acid sequence of the conformational epitope is not homologous to a continuous amino acid sequence of the natural or recombinant protein, the homology being defined by the combination of two criteria:

- the amino acid identity criterion determined by the ratio between the number of amino acids of a peptide according to the invention which are identical to those of a sequence of the same size carried by the natural or recombinant protein, and the total number of amino acids of said peptide. Preferably, the amino acid identity will

preferably not exceed 50%, even 60% or 70% or 80% or even 90%;

- the linkage criterion determined by the ratio between the number of amino acids of a peptide according to the invention which are both identical and are present in the same linkage position as those of a sequence of the same size carried by the natural or recombinant protein, and the total number of amino acids of said peptide. Preferably, the linkage identity will not exceed 70 to 80%.

The term "long-term nonprogressor" is understood to mean HIV-positive subjects characterized from the clinical point of view in that they have not developed AIDS since they were infected over a period of at least ten years, from the biological point of view in that they do not show signs of immunosuppression with in particular a level of CD4 T lymphocytes greater than $600/mm^3$ and finally who are not receiving a particular antiviral treatment.

The expression "mimotope" is understood to mean an epitope which mimics the three-dimensional structure of another epitope by binding to the specific binding site of the same antibody.

The expression "CDR3" is understood to mean the hypervariable region of the linkage of amino acids of the heavy and light immunoglobulin chains which is situated at the level of the specific site for interaction with the epitope.

The expression "conjugate" is understood to mean the combination of the peptide as defined in the invention with any other molecule, by physical or chemical processes, intended to induce or enhance the immunogenicity of the initial peptide.

The expression "immunogenicity" is understood to mean the capacity of a molecular entity, after inoculation into a mammal, to induce a production of antibody specifically directed against this entity.

The expression "polynucleotide" is understood to mean either an RNA sequence, or a DNA sequence, or a cDNA sequence resulting from the reverse transcription of a sequence of natural or synthetic origin, with or without modified bases.

The expression "mucosal route" is understood to mean a mode of administration which brings the pharmaceutical composition directly into contact with the various types of mucous membranes of the body.

The expression "parenteral route" is understood to mean a mode of administration which brings the pharmaceutical composition directly into contact with the internal tissues or organs of the body.

The invention is therefore aimed at any peptide which mimics a conformational epitope of an antigen of the HIV envelope and which is recognized by an antibody obtained from a "long-term nonprogressor" patient and specific for this antigen. A peptide according to the invention may be advantageously represented by one of the 11 sequences as follows

SEQ ID NO: 1 Phe Asn Leu Thr His Phe Leu
SEQ ID NO: 2 Glu Gly Trp His Ala His Thr
SEQ ID NO: 3 Lys Leu Asn Trp Met Phe Thr
SEQ ID NO: 4 Ser Thr Asn Trp Met Phe Thr
SEQ ID NO: 5 Ala Met Pro Leu Pro Tyr Thr Phe
SEQ ID NO: 6 Asp Ser His Thr Pro Gln Arg
SEQ ID NO: 7 Val Ser Phe Thr Pro Ser Phe
SEQ ID NO: 8 His Ala Ala Leu Ser Met Asn Thr His Ala Leu Met
SEQ ID NO: 9 Ala Trp His Glu Ser Arg Ala
SEQ ID NO: 10 Phe Lys Thr Ala Tyr Pro Thr
SEQ ID NO: 11 Ser His Ala Leu Pro Leu Thr Trp Ser Thr Ala Ala

From a combinatorial antibody library obtained in particular from peripheral blood of a subject who has been infected with the HIV virus and belongs to the "long-term nonprogressor" group, characterized in that said subject belonging to this group has been asymptomatic from the clinical point of view for at

least 10 years and shows no biological signs of immunosuppression with in particular a level of CD4 T lymphocytes greater than 600/mm^3, and from a synthetic peptide library, it is possible to identify, by means of mimotopes, novel conformational epitopes present on the HIV envelope, in particular epitopes which may be located outside the V3 loop, such as for example epitopes located in the region of the site for binding to the CD4 receptor, epitopes overlapping the V2 loop and the site for binding to the CD4 receptor or even epitopes overlapping the C2, C3 and V4 regions of Gp120 or epitopes located in the nonimmunodominant regions of Gp41 (clusters I and II). The identification of these mimotopes requires, for their use, a sophisticated technological process, namely:

- the preparation of a combinatorial antibody library which is sufficiently complex to better reflect the natural antibody repertoire of one individual, and advantageously the antibody repertoire of an individual infected with HIV and belonging to the "long-term nonprogressor" group;

- the selection, from this library, of recombinant antibodies specific for antigens of the HIV envelope, in particular expressed by Gp160, Gp120, Gp41 which may be in the form of natural or recombinant, glycosylated or deglycosylated, monomeric or multimeric proteins or finally of proteins combined with each other or otherwise. The selection of specific recombinant antibodies preferably comprises an additional step consisting in measuring the neutralizing activity of these recombinant antibodies toward the viral infection mediated in particular by one or more primary HIV virus isolates. The recombinant antibodies specific for and neutralizing several primary HIV virus isolates will be preferably selected;

- the selection of peptides specific for the recombinant antibodies, from a random synthetic peptide library obtained by molecular recombination, it being possible to carry out said selection by ELISA;

- the characterization of these peptides as being mimotopes of conformational epitopes of HIV in that there is no correspondence between the amino acid sequence of said peptide with any continuous amino acid sequence found in the proteins of the HIV envelope, on the one hand, and, on the other hand, in that this peptide is capable of inhibiting the interaction of the recombinant antibody with the product of the env gene which served for the selection of said recombinant antibody.

It is also possible to carry out the transformation of lymphocytes obtained from a "long-term nonprogressor" patient using, for example, the Epstein-Barr virus (EBV) for the selection of novel monoclonal antibodies specific for the HIV envelope. The method for lymphocyte transformation is well known to a person skilled in the art and results, after several cycles of selection against the antigen of interest, in the production of transformed and immortal lymphocyte clones, each clone producing a single type of monoclonal antibody. These monoclonal antibodies, like the recombinant antibodies derived from the combinatorial library, may also be tested for their neutralizing activity against various primary HIV isolates before being used for the selection of mimotope peptides of the HIV envelope.

To induce or rather enhance the immunogenicity of the peptide mimicking a conformational HIV epitope, the subject of the present invention is also peptides comprising a repetition (2 or more) of the peptide in accordance with the invention. In particular, the coupling of the two identical peptides may be performed, if necessary, by means of an intercalating spacer arm consisting of the linkage of amino acids Gly Pro Gly.

The invention also relates to a combination of various peptides in accordance with the invention, as well as to peptides comprising both repetitions and combinations.

In such cases, the peptides may be joined by covalent bonds or noncovalent bonds. For example, there may be advantageously mentioned the method developed by Posnett et al (J. Biol. Chem. (1988) 263: 1719) which does not alter the three-dimensional structure of the epitope or of the epitopes carried by the peptide and results in the formation of multimers of the same peptide or of different peptides.

In the context in particular of antigenic preparations and of vaccine formulations which are described below, it is also possible to prefer conjugating, through covalent bonding, the peptides of the invention with immunogenic molecules customarily used for making small-sized peptides immunogenic.

The peptides according to the invention may thus be conjugated with known immunogenic proteins such as serum albumins, thyroglobulin, ovalbumin, gelatin, hemocyanin (e.g. Keyhole Limpet Hemocyanin KLH), seroglobulins, tetanus toxoid, diphtheria toxoid, bacterial outer membrane proteins, and the like, but it is also possible to prefer conjugating the peptides with "helper T" epitopes among which there may be chosen in particular the HIV "helper T" epitopes, for example the T1 and p24E epitopes as described in WO 94/29339 (Connaught). The reactions for conjugation with said HIV "helper T" epitopes allow us to obtain compounds whose sequential linkage is p24E-GPG-X-GPG-T1, where:

- p24E symbolizes the amino acid sequence of the epitope p24E, said sequence being placed on the N-terminal side of the peptide according to the invention;
- GPG symbolizes the glycine-proline-glycine linkage;
- X symbolizes the peptide of interest according to the invention which maybe, if necessary, the product of the combination of several identical or different amino acid sequences in accordance with the invention;

- T1 symbolizes the amino acid sequence of the epitope T1, said sequence being placed on the C-terminal side of the peptide according to the invention.

Among these compounds, there may be mentioned the compounds comprising the sequences 12 to 14

SEQ ID NO: 12 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Lys Leu Asn Trp Met Phe Thr Gly Pro Gly Lys Leu Asn Trp Met Phe Thr Gly Pro Gly Iys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

SEQ ID NO: 13 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Ser Thr Asn Trp Met Phe Thr Gly Pro Gly Ser Thr Asn Trp Met Phe Thr Gly Pro Gly Iys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

SEQ ID NO: 14 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Phe Asn Leu Thr His Phe Leu Gly Pro Gly Phe Asn Leu Thr His Phe Leu Gly Pro Gly Lys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

These conjugates can themselves be grafted onto a branched lysine backbone so as to obtain polymers of said conjugates in branched form as described in WO 94/29339 (Connaught), the technical content of said patent being incorporated by reference into the subject-matter of the invention.

The techniques for conjugation are also perfectly known to persons skilled in the art. There may be used, for example, heterobifunctional agents such as SPDP, carbodiimide, glutaraldehyde, biotin/avidin system, and the like.

It is also possible to couple the peptides to lipopolysaccharides, polysaccharides, glycopeptides, muramyl peptide analogs, fatty acids, and the like.

Preferably, the coupling of a peptide with a fatty acid of the type comprising palmitoyl-lysine as described in EP 491628 (Biovector) or (Pam)3 Cys-Ser as described in EP547681 (Merck) for example, the technical content of said patents being incorporated by reference into the subject-matter of the invention.

The methods for operably linking individual peptides by side chains carrying amino acid residues, in order to form an immunogenic conjugate, for example a branched polypeptide polymer, are also well known to persons skilled in the art. By these methods, it is sought to establish bonds on various side chains by one or more types of functional groups in order to obtain a structure in which the peptide structures are covalently linked while being separated by at least one side chain. As functional groups, there may be mentioned the epsilon-amino groups, beta- or gamma-carboxylic groups, thiol (-SH) groups and aromatic rings (for example tyrosine and histidine). Methods for binding polypeptides with the aid of these functional groups are described in Erlanger (1980 Method of Enzymology, 70: 85), Aurameas et al., (1978 Scand. J. Immunol., Vol. 8, suppl. 7, 7-23) and US-A-4 193 795. In addition, it is also possible to use a directed coupling reaction as described in Rodwell et al., (1985 Biotech 3, 889-894). The peptides may also be modified in order to incorporate spacer arms such as hexamethylenediamine or other bifunctional molecules of similar sizes.

The peptides may also be formulated with alum, monophosphoryl Lipid A, pluronics, SAF1, Ribi, trehalose-6,6-dimycolate or other immunostimulatory compounds known to persons skilled in the art to increase the immunogenicity of the peptide to which these compounds are bound.

However, all these methods of conjugation, modification, repetition or combination of peptides in accordance with the invention must observe the original conformation of the peptide as much as possible.

The subject of the present invention is also the DNA fragments encoding the peptides according to the invention and which can be used to produce the peptides by expression of the DNA sequence in an appropriate expression system. By taking into account the degeneracy of the code, persons skilled in the art are perfectly capable of determining the various DNA sequences capable of encoding the various peptides in accordance with the invention.

According to a first aspect of the invention, the expression system is an in vitro expression system for the production of the peptides for their subsequent use, e.g. as diagnostic reagent, as antigenic component or as vaccine component. Such in vitro expression systems or vectors are perfectly known to persons skilled in the art and there may be mentioned by way of example bacteria such as E. coli, eukaryotic cells such as yeasts, in particular S. cerevisiae, baculovirus, in particular propagated on insect cells, and the like.

The subject of the invention is therefore also an expression cassette comprising such a DNA fragment and regulatory sequences allowing the expression of this DNA fragment in an appropriate in vitro expression system.

According to a second aspect of the invention, the expression system is an in vivo expression system for generating a preferably protective immune response in the treated patient. In other words, the expression system, which may be replicative or nonreplicative, will express the peptide in vivo. Persons skilled in the art have such systems at their disposal. By way of preferred examples, there may be mentioned plasmids, in particular naked plasmids, e.g. according to WO-A-90 11092, WO-A-93 19813, WO-A-94 21797 and WO-A-95 20660, poxviruses, such as the vaccinia virus and avian poxviruses (fowlpox, pigeonpox, canarypox, and the like), adenoviruses, and the like.

The subject of the invention is therefore also expression cassettes comprising such a DNA fragment and

the means for regulating expression in the chosen expression system. Its subject is also the expression system or expression vector, comprising such an expression cassette, in particular a plasmid, a poxvirus or an adenovirus, as seen above.

The subject of the invention is finally the use of phages expressing the peptide of interest or a combination of phages expressing the peptides of interest as a diagnostic reagent or as an antigenic or vaccine component.

The invention also relates to the use of at least one peptide in accordance with the invention in combination or otherwise with at least one recombinant vector in accordance with the invention for the preparation of a pharmaceutical composition intended for preventing or curing an HIV virus-related condition. A composition according to the invention may comprise preparations which may be in the form of creams, powders which are freeze-dried or otherwise, solutions, suspensions, for administrations by the mucosal route such as the oral, nasal, rectal, genital or cutaneous route for example. For parenteral administrations such as intradermal, subcutaneous, intramuscular, intravenous, intraarterial, intralymphatic or intraperitoneal administration, for example, the sterile injectable preparations may be, depending on the cases, in the form of solutions, suspensions or emulsions. In addition to the active ingredient(s), in accordance with the subject of the invention, the preparations may contain excipients and/or stabilizing agents suited to the mode of administration.

The preparations intended for a vaccine use may also contain adjuvants or be incorporated into delivery systems compatible with a use in human medicine. There may be mentioned in particular the use of adjuvants such as Alum (aluminum phosphate or hydroxide or the mixture of the two) conventionally incorporated into vaccines, incomplete Freund's adjuvant, mono-

phosphorylated lipid A (MPL), QS21, Polyphosphazene, muramyl dipeptide (MDP) or its derivatives, the use of a system for delivering the antigen such as the emulsions (MF59, SAF1, RIBI, SB 62, SB 26), ISCOMS, liposomes, microspheres composed of PLGA polymers having a well calibrated diameter, or optionally pseudovirions.

The doses and routes for administration of these pharmaceutical compositions will be determined taking into account the nature of the composition, the level of expression of the peptide of interest by the recombinant vector if it is included in the preparation, the age, sex and weight of the individual receiving the preparation. Account will also be taken of the relative importance of the carrier molecule in the conjugate if it is included in the composition.

Taking into account all these factors which are known and recognized by persons skilled in the art, the peptide doses administered may be up to 1 to 5 mg but more generally will be between 5 µg and 1 mg per injection, preferably 50 to 500 µg. The recombinant vector encoding the peptide of interest may be administered or used to transfect or infect the cells of interest at a minimum dose of $10^{3.5}$ infectious units (pfu or plaque forming unit). Preferably, the recombinant vector will be used in a dose range going from 10^4 to 10^{10} pfu depending on the efficiency of expression of the peptide by this vector and in particular in a dose range going from 10^6 to 10^9 pfu, for example. When the pharmaceutical composition comprises several recombinant vectors encoding different peptides of interest, it is clearly understood that these same ranges of doses may be applied to these combinations. Persons skilled in the art will be able to refer to the clinical protocols and trials using preparations based on recombinant vectors, in particular recombinant poxviruses or recombinant adenoviruses, already carried out in humans in order to

agree on the appropriate number of pfu which the pharmaceutical composition should contain.

When the pharmaceutical composition comprises a plasmid containing the system for expressing the peptide of interest, account will be taken, in dosing this composition, of the level of immune response which this composition is capable of inducing, which should be at least equal to that of the intact or modified peptide and/or of the level of expression of the peptide induced by the plasmid in the cells of the body which should be as close as possible to that obtained by the recombinant vectors already mentioned. For example, the quantities of plasmids contained in the pharmaceutical compositions may be in ranges going from 1 µg to 100 mg, preferably between 0.1 mg to 10 mg. Persons skilled in the art will be able to refer to the clinical protocols and trials already carried out in humans, using plasmid DNA preparations in order to agree on the plasmid dose which the pharmaceutical composition should contain.

For the prevention of HIV infection, the pharmaceutical composition may be administered in a single dose or in divided doses in order to reach the desired level of response comprising in particular the level and the quality of the specific antibody and/or specific cell-mediated response desired, and characterized in that it ensures protection of the individual against accidental infection. To achieve this objective, it may be necessary, in addition to the composition of the preparation and the route of administration chosen, to observe the periods allowed between each injection, which may be preferably 1 month, 2 months or 6 months and/or to use in combination or alternately for the duration of the medical, in particular vaccine, protocol different pharmaceutical compositions relating to the peptide, to the recombinant vector, to the plasmid of interest or even to the phages expressing the peptide(s) of interest which persons skilled in the art are capable

of controlling. It may also be necessary, in order to maintain the level of protection, to perform booster injections at regular intervals.

For the treatment of HIV-related infection, the pharmaceutical, in particular vaccine, composition may be administered in a single dose or in divided doses and at intervals which may be very short, in particular within periods of less than one week, in order to reach the desired level of response, in particular that which makes it possible to observe the absence of the HIV virus in the blood by the PCR test. If necessary, the pharmaceutical composition comprising the peptide, the vector, the plasmid of interest or even the phages expressing the peptide(s) of interest may be combined or used alternately with the conventional treatments for this condition, comprising in particular antiviral mono-, bi- or tritherapy.

Whether for the prevention or the treatment of HIV infection, it may also be useful to use a pharmaceutical composition comprising one or more peptides of interest, the corresponding recombinant vector(s) of interest as well as the plasmid(s) of interest or even the bacteriophages expressing the peptide(s) of interest in order to stimulate the cells of the immune system of the patient in vitro or ex vivo and to then reinject them into the body of the individual. This method was in particular developed in the immunotherapeutic treatment of cancer.

The subject of the invention is finally the use of the peptides of interest as reagent for the diagnosis of HIV infection which makes it possible in particular to identify subjects more resistant to the infection also called "long-term nonprogressors" or on the contrary to identify the infected subjects more likely to rapidly develop AIDS. For the first time, novel conformational epitopes of the HIV envelope have been defined. These peptides can therefore be used, for diagnostic purposes, to preferably test for neutralizing antibodies for primary HIV isolates, which

very often recognize conformational epitopes and thus make it possible to distinguish the "long-term nonprogressor" individuals (possessing neutralizing antibodies) from those which are likely to rapidly develop AIDS if no treatment, in particular antiviral treatment, is rapidly introduced (possessing no neutralizing antibodies).

The subject of the present invention is therefore also a method for the diagnosis of HIV infection and/or of susceptibility of infected subjects to rapidly develop AIDS, said method being preferably based on the analysis of the humoral response. For the analysis of the humoral response, immunoenzymatic, radioimmunological or Western blotting methods which are well known to persons skilled in the art, such as for example the ELISA, RIA, RIPA or IRMA methods, may be used.

Description of the figures

Figures 1 to 5 represent, as a function of their dilution, the curves for the binding of phages respectively expressing the peptide sequences SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 to a recombinant antibody specific for Ggp 160 and derived from the combinatorial antibody library produced from lymphocytes of a "long-term nonprogressor" subject (■) and to an antibody IgG not specific for the HIV envelope antigen (●), these two antibodies being previously bound to ELISA plates. The intensity of the binding is proportional to the optical density (OD) value obtained by ELISA.

The present invention is described in greater detail below with the aid of the additional description which follows, which refers to examples of selection of antibodies directed against the HIV envelope, selection of peptides according to the invention, synthesis of peptides according to the invention, induction of antibodies specific for peptides according to the invention, vaccine compositions according to the invention and use of peptides according to the

invention for the diagnosis of HIV infection. It goes without saying, however, that these examples are given by way of illustration of the subject of the invention and do not in any manner constitute a limitation thereto.

Example 1: Selection of peptides

The manufacture of recombinant antibodies using molecular biology methods have developed substantially over the last ten years and are now well known to persons skilled in the art. It is also known that the specificity of a recombinant antibody is essentially carried by the CDR3s of the light and heavy chains. Knowledge of the amino acid linkage which represents CDR3 and of the structure of the backbone of the heavy and light chains of a given antibody is sufficient for persons skilled in the art to be able to reproduce and reconstitute an equivalent recombinant antibody having the same characteristics of recognizing said antibody.

The sequences encoding the portions of heavy and light chains of the Fab molecules selected may be isolated and synthesized, and cloned into any vector or replicon allowing their expression.

Any appropriate expression system may be used, for example bacteria, yeasts, insect, amphibian and mammalian cells. The systems for expression in bacteria include those described in Chang et al. (1978) Nature 275: 615, Goeddel et al. (1979) Nature 281: 544, Goeddel et al. (1980) Nucleic Acids Res. 8: 4057, EP-A-36,776, US-A-4,551,433, deBoer et al. (1983) Proc. Natl. Acad. Sci. USA 80: 21-25, and Siebenlist et al. (1980) Cell 20: 269. The systems for expression in yeasts include those described in Hinnen et al. (1978) Proc. Natl. Acad. Sci. USA 75: 1929, Ito et al. (1983) J. Bacteriol. 153: 163, Kurtz et al. (1986) Mol. Cell. Biol. 6: 142, Kunze et al. (1985) J. Basic Microbiol. 25: 141, Gleeson et al. (1986) J. Gen. Microbiol. 132: 3459, Roggenkamp et al. (1986) Mol. Gen. Genet. 202: 302, Das et al. (1984) J. Bacteriol. 158: 1165, De Louvencourt et al. (1983) J. Bacteriol. 154: 737, Van

den Berg et al. (1990) Bio/Technology 8: 135, Kunze et al. (1985) J. Basic Microbiol. 25: 141, Cregg et al. (1985) Mol. Cell. Biol. 5: 3376, US-A-4,837,148 and 4,929,555, Beach et al. (1981) Nature 300: 706, Davidow et al. (1985) Curr. Genet. 10: 380, Gaillardin et al. (1985) Curr. Genet. 10: 49, Ballance et al. (1983) Biochem. Biophys. Res. Commun. 112: 284-289, Tilburn et al. (1983) Gene 26: 205-221, Yelton et al. (1984) Proc. Natl. Acad. Sci. USA 81: 1470-1474, Kelly et al. (1985) EMBO J. 4: 475479; EP-A-244,234 and WO-A-91/00357. The expression of heterologous genes in insects may be carried out as described in US-A-4,745,051, EP-A-127,839 and EP-A-155,476, Vlak et al. (1988) J. Gen. Virol. 69: 765-776, Miller et al. (1988) Ann. Rev. Microbiol. 42: 177, Carbonell et al. (1988) Gene 73: 409, Maeda et al. (1985) Nature 315: 592-594, Lebacq-Verheyden et al. (1988) Mol. Cell. Biol. 8: 3129, Smith et al. (1985) Proc. Natl. Acad. Sci. USA 82: 8404, Miyajima et al. (1987) Gene 58: 273, and Martin et al. (1988) DNA 7: 99. Numerous strains and variants of baculoviruses and permissive insect cells are described in Luckow et al. (1988) Bio/Technology 6: 47-55, Miller et al. (1986) GENERIC ENGINEERING, Setlow, J.K. et al. Eds. Vol. 8, Plenum Publishing pp. 277-279, and Maeda et al. (1985) Nature 315: 592-594. The expression in mammalian cells may be carried out as described in Dijkema et al. (1985) EMBO J. 4: 761, Gorman et al. (1982) Proc. Natl. Acad. Sci. USA 79: 6777, Boshart et al. (1985) Cell 41: 521, and US-A-4,399,216. Reference may also be made to Ham et al. (1979) Meth. Enz. 58: 44, Barnes et al. (1980) Anal. Biochem. 102: 255, US-A-4,767,704, 4,657,866; 4,927,762; 4,560,655; US patent RE 30,985, WO-A-90/103430 and WO-A-87/00195.

The peripheral blood lymphocytes obtained from a subject who has been infected with the HIV virus and who belongs to the "long-term nonprogressor" group are used to produce the combinatorial antibody library. The cDNA of the lymphocytes is obtained from the RNA using a method developed by Sodoyer R. et al. (1997) Human

Antibodies 8: 37. The heavy and light chain library is constructed using the phagemids pVH (pM 831) and pVL (pM452). The two libraries are then combined in a "Random" manner by subcloning the VL genes into the heavy chain library. The phagemid library obtained is then infected with the helper phage M13 VCS thus allowing the expression of the Fab's at the surface of the phages. After selection of the phages expressing the Fab's at their surface by "panning" against the Gp 160 protein, the nucleotide sequence of the recombinant Fab's expressed by the positive isolates and in particular the CDR3 sequences carried by the fragments of heavy and light chains, is determined.

One of the recombinant antibodies specific for Gp 160 obtained from this combinatorial library is then used to select the peptide sequences SEQ NO: 1 to SEQ NO: 11 from a commercially available phage library randomly expressing peptides (pHD7, NEB) by carrying out the procedure in the following manner:

1.4×10^{11} phages are incubated with 30 or 300 ng of the recombinant antibody according to example 1 in 200 µl of PBS-0.1% tween 20 for 20 min at 20°C. The mixture is transferred into a tube containing 50 µl of G protein coupled to sepharose beads previously equilibrated for 1 hour in 1 ml of PBS-0.1% tween 20 containing 5% of skimmed milk. After another incubation of 20 min, the beads are centrifuged and washed 3 times with 1 ml of PBS-0.1% tween 20. On the 4th washing, the beads are taken up in PBS-0.1% tween 20 containing 5% of skimmed milk, incubated for 10 min, centrifuged and rinsed again 3 times with PBS-0.1% tween 20. After the last centrifugation, the beads are taken up in 1 ml of 0.2 M glycine-HCl, pH = 2.2, incubated for 10 min at 20°C and centrifuged. The supernatant is transferred into a tube containing 60 µl of 2 M Tris base, pH = 7.5, so as to neutralize the solution and then incubated with 2 ml of E. coli 7118 bacteria in the exponential growth phase for 15 min. The culture volume was adjusted to 100 ml with LB medium, and the incubation was continued for

4 h at 37°C. After a centrifugation intended to remove the bacteria, the phages are precipitated by addition of 25 ml of 20% PEG-2.5 M NaCl in the culture supernatant overnight at 4°C. After centrifugation (10 000 rpm, 20 min, 4°C), the phage pellet is taken up in 1 ml of PBS-0.1% tween 20-1% skimmed milk, and the titer determined. The screening is complete when the whole process has been repeated 3 to 4 times.

To complete the selection of the phages expressing the peptide sequences 1 to 11, they were also tested by ELISA as follows:

0.2 mg of recombinant antibody specific for Gp 160 or of antibody which has no specificity for an antigen of the HIV envelope called "Ig control", diluted in 50 µl of PBS, is deposited in each well of an ELISA plate followed by incubation overnight at 4°C. After replacing the antibody solution with 0.1 ml of PBS-0.1% tween 20 (PBST) containing 5% of skimmed milk and incubating for 1 h at 37°C, and preparing a 2-fold serial dilution on the phages expressing the peptide sequences SEQ ID NO: 1 to SEQ ID NO: 11 in PBST-1% skimmed milk, the various dilutions prepared are distributed into the wells sensitized either with the specific recombinant antibody according to example 1, or with the Ig control. After 2 h at 37°C, the phages are removed by aspiration of the dilutions and then the wells are washed 10 times with 0.2 ml of PBST. The wells are then incubated for 1 h at 37°C with a 1:1000 solution in PBST-1% milk of a biotinylated antibody directed against the phage fd (SIGMA). After another series of washes in PBST-1% milk, a streptavidin-peroxidase (SIGMA) complex diluted to 1:2000 in PBST is added to each well, followed by incubation for 1 h at room temperature and washes in PBST-1% milk. The enzymatic activity of peroxidase is conventionally visualized by addition of an OPD solution diluted to 1 mg/ml in sodium citrate buffer. The intensity of the color of the OPD solution is then measured on a spectrophotometer, and then the OD (optical density)

curves are established as a function of the phage dilution. The curves of figures 1 to 5 show, by way of example, that the phages expressing the peptide sequences SEQ ID NO: 7 to SEQ ID NO: 11 indeed bind to the specific recombinant antibody (OD $\geq$ 0.6 observed for at least one phage dilution) whereas there is no significant binding to the Ig control (OD $\leq$ 0.3 whatever the phage dilution tested in the dilution series going from 5×10^{11} phages/well to 4.8×10^{8} phages/well). The positive phages, that is to say those which bind specifically to the specific recombinant antibody are amplified in E. coli. Mini preparations of phage DNA are prepared according to the procedures described in the manuals by Maniatis, the DNA is sequenced with the aid of an automated sequencer from which the sequence of the peptide expressed by the phage is deduced.

Example 2: Synthesis of peptides

The peptides of SEQ ID NO: 1 to SEQ ID NO: 11 are synthesized in solid phase by referring to the methods developed in the manuals Solid phase peptide synthesis: a practical approach, IRL Press, Oxford, 1989 and Solid phase peptide synthesis, second edition, published by Pierce Chemical Company, 1984.

The α-amino function of the amino acids is protected by introducing a t-butyloxycarbonyl (t-boc) group thus allowing coupling through the carboxyl function of the amino acid to an active chloromethylated resin. After binding to the resin, the amine function is "deprotected" by the action of trifluoroacetic acid followed by a step of neutralizing with triethylamine. The amine function thus released then undergoes a reaction for coupling with another amino acid in the form of a t-boc derivative via carbodiimides. This method is used by the ABI (Applied Biosystem Inc) 430A automated device which thus performs the automated synthesis of peptides. At the end of the synthesis, the peptide is detached from the resin by the action of hydrofluoric acid. The extract

is then purified by reversed-phase HPLC using a semipreparative column of the Vydac C4 type and an acetonitrile gradient ranging from 15 to 55% in a 0.1% trifluoroacetic acid solution. Liquid chromatography is programed for a period of 40 min with a flow rate of 2 ml/min. The level of purity of the peptides is checked by analytical chromatography and exceeds 95%.

Example 3: Induction of specific antibodies

1) Induction of antibodies in guineapigs and rabbits

The peptides of SEQ ID NO: 1 to 6, presented on phages, are injected into guineapigs and rabbits: 2 injections of 100 microliters by the intravenous route, at an interval of 3 weeks followed by a final bleeding 15 days after the 2nd injection. The sera are tested by ELISA against gp160 and a reaction is observed against the glycoprotein. These peptides are therefore capable of inducing a response against gp160.

2) Induction of antibodies in mice

The various isolates of phages expressing the peptides defined by SEQ ID NO: 7 to SEQ ID NO: 11 are purified on a cesium chloride gradient. 5 groups of BaLB/c mice were then identified. Each group was immunized by the intraperitoneal route, 3 times at an interval of 3 weeks with only one type of purified isolate expressing either SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 at the rate of 10^{12} purified phages per injection. To compare the antibody responses, we introduced 2 groups of additional mice, the first group receiving 3 injections of 10^{12} phages expressing peptides not mimicking conformational epitopes of the HIV envelope (irrelevant phages), the second receiving 3 injections of 5 µg of Gp160 protein. We also introduced an 8th group of mice which received a mixture of phage isolates, comprising, in equal parts, phages expressing SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11. A few mice in this group as well as a few mice in the group immunized with the irrelevant phages received a 4th injection of 5 µg of Gp 160, 3

weeks after the 3rd injection. The analysis of the specific antibody response is performed on the serum of the mice in each group collected 15 days after each injection. The analysis of the specific antibody response comprises the detection of anti-gp 160 antibodies by ELISA with the aid of plates sensitized with the Gp 160 protein using a procedure similar to that described in example 1 and the test for neutralizing antibodies. For the test for neutralizing antibodies, the serum dilution which prevents the formation of syncytia in 50% of the microwells infected with 10 $CCID_{50}$ of an HIV virus strain is determined. After decomplementizing the sera and preparing a 2-fold serial dilution in RPMI medium, 500 µl of the HIV virus suspension titrating $10^{2.5}$ $CCID_{50}$/ml are mixed with 500 µl of various dilutions of sera. After incubating for 2 hours at 37°C, the mixture is deposited in a volume of 100 µl on CEMss cells previously attached in microwells (6 microwells/dilution of serum). After 1 hour of contact at 37°C with the CEMss cells, the mixture is aspirated and replaced with culture medium. After 7 and 14 days of incubation, the cultures are examined under a microscope for the enumeration of the syncytia. The titer neutralizing by 50% is determined according to the Spearman and Kärber method. A good production of antibodies is observed in the mice in the 8th group which was immunized with the mixture of isolates of phages expressing the various peptide sequences (SEQ ID NO: 7 to SEQ ID NO: 11)

Example 4: Vaccine formulations

A peptide having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 and produced by chemical synthesis according to example 2 or obtained from the product of expression of a recombinant vector and in particular of a recombinant baculovirus using the techniques developed by Smith et al (USA 4,745,051). Water-in-oil emulsions were then prepared using squalene as constituent of the organic phase, tween 80 or a mixture of tween 80 and SPAN as

surfactant, the aqueous phase containing the peptide solution. When the hydrophobicity of the peptide is very high, oil-in-water emulsions are prepared in which the peptide will be associated with the organic phase. If necessary, immunostimulants such as QS 21, derivatives of MPL, or any other adjuvant are incorporated into the preparation of these emulsions. This formulation is used for the preparation of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 5: Vaccine formulations

A vaccine formulation based on liposomes is prepared which comprises a peptide having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 and produced by chemical synthesis according to example 2 by reference to manuals such as "Liposomes as Drug Carriers" published by G. Gregoriadis, 1988, or to volumes 1 to 3 of "Liposome Technology published by G. Gregoriadis, 1984. This formulation is used for the preparation of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 6: Vaccine formulations

A vaccine formulation based on ISCOMs is prepared which contains a peptide having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 and produced by chemical synthesis according to example 2 by reference to B Morein et al, 1984, Nature 308:457 or B Morein et al Immunology toDay, 1987, 8(11):333.

Example 7: Vaccine formulations

A formulation based on microparticles is prepared which comprises a peptide having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 and produced by chemical synthesis according to example 2 mimicking a conformational epitope of the HIV envelope. For the preparation of microparticles or of nanoparticles, many synthetic or natural polymers are used such as the methyl metacrylate polymer (Troster S.D. et al, 1992, J. Microencaps. 9:19) but

often poly(d,l-lactide-co-glycolide) also called PLGA is the referent because of its biodegradability, its safety and its already old applications in the medical field. The microparticles of PLGA, loaded with peptides, are prepared in particular by a water-in-oil-in-water double emulsion. The peptide is solubilized in aqueous phase and then emulsified in a solution of PLGA in the organic phase such as dichloromethane. The water-in-oil emulsion is obtained by stirring the peptide solution at high speed in the organic solution of PLGA. A second aqueous phase containing an appropriate concentration of surfactant such as polyvinyl alcohol is then added to the first emulsion in order to thus produce the double emulsion. Other surfactants are also used such as bile salts or poly(oxyethylene glycerol monoleate) in order to stabilize the double emulsion (Rafati H et al., 1997, Vaccine 15: 1888). After stirring overnight in order to allow evaporation of the solvent, the microparticles of PLGA are washed several times in distilled water and then freeze-dried and stored at 5°C. This formulation is used for the preparation of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 8: Vaccine composition

The peptides which contain less than 20 amino acids may be weakly immunogenic. To increase the immunogenicity of the peptides having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 and produced by chemical synthesis according to example 2, a vaccine formulation is prepared which is based on polymers of the same peptide or of different peptides, in the form of octamers comprising a branched polylysine structure containing 8 side arms onto which the same peptide or different peptides according to example 1 are attached using the method developed by Posnett D.N et al. (1988) J. Biol. Chem. 263: 1719. This formulation is used for the preparation

of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 9: Vaccine composition

A vaccine composition is prepared which comprises a peptide having one of the sequences SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 4 described in example 1 and produced by chemical synthesis according to example 2, according to example 2 flanked by the 2 helper T epitopes P24E and T of the HIV virus, these 2 epitopes playing the role of carrier molecule and thus enhancing the immunogenicity of the peptide. The reaction for coupling the peptide to these 2 helper T epitopes occurs in 2 stages according to conventional methods well known to persons skilled in the art. In a first stage, the coupling of the N-terminal portion of the peptide with the C-terminal portion of the peptide sequence representing the epitope p24E is carried out by intercalating a spacer consisting of the glycine-proline-glycine sequence. In a second stage, the coupling of the C-terminal portion of the intermediate product with the N-terminal portion of the peptide sequence representing the T1 epitope is then carried out by intercalating the same glycine-proline-glycine sequence in order to obtain the final product. This formulation is used for the preparation of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 10: Vaccine composition comprising a lipopeptide

A vaccine formulation is prepared which comprises a peptide having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 and produced by chemical synthesis according to example 2 coupled to one or more chains among derived from fatty acids, which N_{ε}-palmitoyllysine, N,N-dipalmitoyllysine, pimelautide, trimexautide or to a steroid group among which N_{ε}[(cholest-5-enyl-3-oxy)acetyl)]lysine or (cholest-5-enyl-3-oxy)acetic acid according to the method described in patent EP0491628 (INSERM) so as to

obtain a lipopeptide. This formulation is used for the preparation of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 11: Expression of the peptides by poxviruses

A vaccine composition is prepared which comprises a recombinant poxvirus encoding a peptide having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 mimicking a conformational epitope of the HIV envelope. The recombinant poxviruses are obtained by homologous recombination from chicken embryo cells infected with the poxviruses and cotransfected with plasmids containing an expression cassette, flanked at the ends with DNA sequences homologous to those of nonessential regions of the poxvirus DNA, and containing, under the control of poxvirus promoters (H6, I3L), the polynucleotide which encodes the peptide according to example 2 using the methods described in patents US 4,769,330, 4,772,848, 4,603,112, 5,100,587, 5,179,993 and 5,863,542. These recombinant poxviruses are used for the preparation of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 12: Expression of several peptides by a poxvirus

A vaccine composition is prepared which comprises a recombinant poxvirus encoding several peptides having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 mimicking several conformational epitopes of the HIV envelope. The use and the preparation of recombinant vectors encoding several epitopes is well known to persons skilled in the art (Toes RE et al. (1997) Proc. Natl. Acad. Sci. USA 94: 14660, Thomson SA et al. (1996) J. Immunol. 157: 822) and is also applicable to the preparation of recombinant poxviruses encoding multiple mimotopes. A vaccine composition is in particular prepared which comprises a recombinant canaripox (recombinant ALVAC) encoding multiple mimotopes of the HIV envelope. These recombinant poxviruses are used for the preparation of

a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 13: Combinations of peptides

A vaccine composition is prepared which comprises several peptides having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 using the various modes of preparation described in examples 2 to 12 mimicking several conformational epitopes of the HIV envelope. These various compositions are used for the preparation of a vaccine composition intended for the prevention or treatment of HIV virus infection.

Example 13: Diagnosis

A detection of antibodies specific for HIV is carried out by ELISA using one or more peptides having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described in example 1 and produced by chemical synthesis according to example 2 for the diagnosis of HIV infection using a biological sample as starting material. In general, a sample of physiological fluid (blood, plasma, serum) is collected, which sample is then caused to react in the presence of a peptide according to the invention.

To do this, the peptide itself is used as diagnostic reagent. Use is generally made of

- either an indirect diagnostic test, of the ELISA type, in which the peptide attached to a support (well) is brought into contact with the sample to be tested, while the visualization of the antigen-antibody attachment is produced by a labeled anti-Ig.
- or a competition or displacement test in which a peptide according to the invention, and a labeled antibody specific for the peptide are used. The peptide is also attached to a solid support such as wells or strips. In the competition test, the peptide is simultaneously brought into contact with the sample (antibody of the sample) and with a labeled antibody specific for the peptide.

Peroxidase-labeled antibodies are generally used.

In the competition or displacement test, monoclonal or polyclonal antibodies or recombinant antibodies specific for the peptide according to the invention, which are sometimes Fab or $F(ab)_2$ fragments and in particular those described in the invention, are generally used.

Example 14: Diagnosis

A detection of antibodies specific for HIV is carried out by immunochromatography using one or more peptides according to the invention for the diagnosis of the HIV infection using a biological sample as starting material.

In this case, the peptide according to the invention is attached to a support of the strip type and reference is made to the article by Robert F.N Zurk et al., Clin. Chem. 31/7, 1144-1150 (1985) as well as to patents or patent applications WO-A-88/08 534, WO-A-91/12528, EP-A-291 176, EP-A-299 428, EP-A-291 194, EP-A-284 232, US-A-5 120 643, US-A-5 030 558, US-A-5 266 497, US-A-4 740 468, US-A-5 266 497, US-A-4 855 240, US-A-5 451 504, US-A-5 141 850, US-A-5 232 835 and US-A-5 238 652 in order to carry out the method.

Example 15: Diagnosis

Study of the lymphoproliferative response specific to one or more peptides according to the invention for the diagnosis of HIV virus infection using a biological sample as starting material.

The patient's blood is collected on a heparinized tube. The lymphocytes are then separated by centrifugation on Ficoll hypaque and then distributed into sterile 96-well microplates at the rate of 2×10^5 cells per round-bottomed well in a final volume of 200 µl of complete culture medium (RPMI 1640 supplemented with 25 mM HEPES, 2 mM L-glutamine, 50 U/ml of penicillin, 50 µg/ml of streptomycin and 5% of decomplementized AB serum) and brought into contact with variable concentrations of one or more peptides having one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11 described

in example 1 and produced by chemical synthesis according to example 2 (concentrations ranging from 1 ng/ml to 50 µg/ml). Each peptide concentration is tested in triplicate so as to better eliminate the biological variations. Multiple combinations of peptides may also be tested in the concentration range indicated, for example a combination resulting from the association of a mimotope of the envelope with a mimotope of the nucleocapsid in the concentration range indicated. After 5 days of culture at 37°C under 5% CO2, 0.5 µci of tritiated thymidine is added to each well. After another incubation of 16 hours, the cellular DNA of each culture well is recovered on filters after ethanol precipitation and the rate of incorporation of tritiated thymidine is measured with the aid of a liquid scintillation counter which reflects the intensity of the lymphoproliferative response. The results are expressed in the form of a stimulation index (mean of the cpm values for the lymphocyte culture wells containing a given concentration of peptide/mean of the cpm values for the lymphocyte culture wells without peptide). The lymphoproliferative response is considered to be positive when the stimulation index is greater than 3.

Claims

1. Peptide for the prevention or therapeutic treatment of HIV virus infection capable of interacting with an antibody specific for an antigen of the envelope of said virus and obtained from an HIV-positive patient belonging to the "long-term nonprogressor" group, comprising an amino acid sequence which mimics a conformational epitope of an antigen of said envelope without, however, corresponding to a continuous amino acid sequence of this antigen.

2. Peptide according to claim 1, according to which the antigen of the envelope is represented by the envelope protein gp160.

3. Peptide according to claim 1 or 2, characterized in that this peptide may comprise the sequences 1 to 11

SEQ ID NO: 1 Phe Asn Leu Thr His Phe Leu
SEQ ID NO: 2 Glu Gly Trp His Ala His Thr
SEQ ID NO: 3 Lys Leu Asn Trp Met Phe Thr
SEQ ID NO: 4 Ser Thr Asn Trp Met Phe Thr
SEQ ID NO: 5 Ala Met Pro Leu Pro Tyr Thr Phe
SEQ ID NO: 6 Asp Ser His Thr Pro Gln Arg
SEQ ID NO: 7 Val Ser Phe Thr Pro Ser Phe
SEQ ID NO: 8 His Ala Ala Leu Ser Met Asn Thr His Ala Leu Met
SEQ ID NO: 9 Ala Trp His Glu Ser Arg Ala
SEQ ID NO: 10 Phe Lys Thr Ala Tyr Pro Thr
SEQ ID NO: 11 Ser His Ala Leu Pro Leu Thr Trp Ser Thr Ala Ala

4. Peptide comprising the linkage of at least two peptides according to one of claims 1 to 3.

5. Peptide according to claim 4, comprising the duplication of identical peptides.

6. Peptide according to claim 5, in which the coupling of the two peptides occurs by means of a "spacer" arm consisting of the amino acid sequence Gly Pro Gly

7. Conjugate comprising at least one peptide according to one of claims 1 to 6, bound to a carrier

molecule in order to induce or enhance the immunogenicity of said peptide.

8. Conjugate according to claim 7, according to which the carrier molecule comprises at least one helper T epitope of the HIV virus.

9. Conjugate according to claim 8, whose carrier molecule comprises the p24E and T1 epitope of the HIV virus

10. Conjugate according to claim 9, comprising a peptide resulting from the duplication of the sequences NO: 1, NO: 3 or NO: 4 according to claim 6, said peptide being bound on the N-terminal side to the p24E epitope and on the C-terminal side to the T1 epitope by means of 2 "spacer" arms.

11. Conjugate according to claim 10, characterized in that the 2 spacer arms are identical and consist of the linkage Gly Pro Gly and in that it comprises any one of the sequences 12 to 14

SEQ ID NO: 12 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Lys Leu Asn Trp Met Phe Thr Gly Pro Gly Lys Leu Asn Trp Met Phe Thr Gly Pro Gly Iys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

SEQ ID NO: 13 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Ser Thr Asn Trp Met Phe Thr Gly Pro Gly Ser Thr Asn Trp Met Phe Thr Gly Pro Gly Iys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

SEQ ID NO: 14 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Gly Pro Gly Phe Asn Leu Thr His Phe Leu Gly Pro Gly Phe Asn Leu Thr His Phe Leu Gly Pro Gly Lys Gln Ile Ile Asn Met Trp Gln Glu Val Glu Lys Ala Met Tyr Ala

12. Recombinant vector comprising a functional expression cassette allowing the expression of a

polynucleotide encoding a peptide according to one of claims 1 to 11

13. Recombinant vector according to claim 12, characterized in that it is an adenovirus, a poxvirus, a baculovirus, a bacteriophage or a plasmid.

14. Therapeutic or prophylactic composition for HIV infection, in particular intended for vaccine use, whose active ingredient comprises a peptide according to one of claims 1 to 11 and/or a recombinant vector encoding said peptide according to either of claims 12 and 13.

15. Composition according to claim 14, whose active ingredient is in the form of a formulation combined with a compatible adjuvant for administration of an effective dose by the mucosal or parenteral route.

16. Use of a peptide according to one of claims 1 to 11 and/or of a recombinant vector according to either of claims 12 and 13 as reagent for the diagnosis of HIV, said diagnosis comprising the evaluation, from a blood sample, of the humoral and/or cell-mediated response specific for this peptide.

17. Use of a peptide according to one of claims 1 to 11 and/or of a recombinant vector according to either of claims 12 and 13 as reagent for the diagnosis of the susceptibility of subjects infected with the HIV virus to rapidly develop AIDS.

18. Use of a peptide according to one of claims 1 to 11 and/or of a recombinant vector according to either of claims 12 and 13 for the preparation of a therapeutic or prophylactic composition intended for the treatment or prevention of HIV infection.

19. Use of a peptide according to one of claims 1 to 11 and/or of a recombinant vector according to either of claims 12 and 13 for stimulating *in vitro* cells of the immune system of an individual, said cells then being intended to be reinjected into the body of the individual after stimulation.

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(54) Title: HIV VIRUS MIMOTOPES

(54) Titre: MIMOTOPES DU VIRUS HIV

(57) Abstract

The invention concerns a peptide for therapeutic or prophylactic treatment of HIV infection, capable of reacting with an antibody specific of the HIV virus envelope and derived from a HIV positive patient belonging to the long term non–progressor group, comprising an amino acid sequence mimicking an antigen conformational epitope of said virus envelope without however corresponding to a continuous sequence of said antigen and capable of containing optionally one of the 11 amino acid sequences as specified in the description. The invention also concerns a peptide conjugate obtained by combining the peptide with a carrier molecule for reinforcing said peptide immunogenicity. The invention further concerns a recombinant vector comprising a functional expression cassette for expressing a polynucleotide coding for said peptide. The invention likewise concerns a therapeutic or prophylactic composition for HIV infection, in particular for vaccinal use, whereof the principle comprises said peptide, or as the case may be said peptide conjugate and/or a recombinant vector coding for said peptide. Finally the invention concerns the use of said peptide as reagent for diagnosing HIV infection and/or the proneness of subjects in contact with the virus for rapidly developing AIDS.

(57) Abrégé

Peptide pour le traitement thérapeutique ou prophylactique de l'infection à HIV, capable de réagir avec un anticorps spécifique de l'enveloppe du virus HIV et provenant d'un patient HIV positif appartenant au groupe des "long term non progressor", comprenant une séquence en acides aminés qui mime un épitope conformationnel d'un antigène de l'enveloppe dudit virus sans toutefois correspondre à une séquence continue d'acides aminés de cet antigène et pouvant contenir au choix l'une des 11 séquences en acides aminés telles que spécifiées dans l'invention. Conjugué du peptide selon l'invention résultant de l'association du peptide à une molécule porteuse pour renforcer l'immunogénicité dudit peptide. Vecteur recombinant comprenant une cassette d'expression fonctionnelle permettant l'expression d'un poly nucléotide codant pour un peptide selon l'invention. Composition thérapeutique ou prophylactique de l'infection à HIV, notamment destiné à un usage vaccinal, dont le principe comprend un peptide selon l'invention, le cas échéant un conjugué de ce peptide et/ou un vecteur recombinant codant pour ledit peptide. Utilisation d'un peptide selon l'invention en tant que réactif pour le diagnostic de l'infection à HIV et/ou de la susceptibilité des sujets en contact avec le virus à développer rapidement un SIDA.

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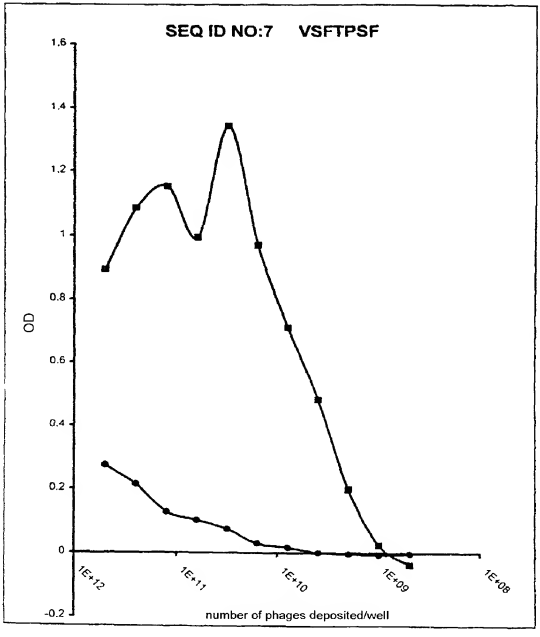

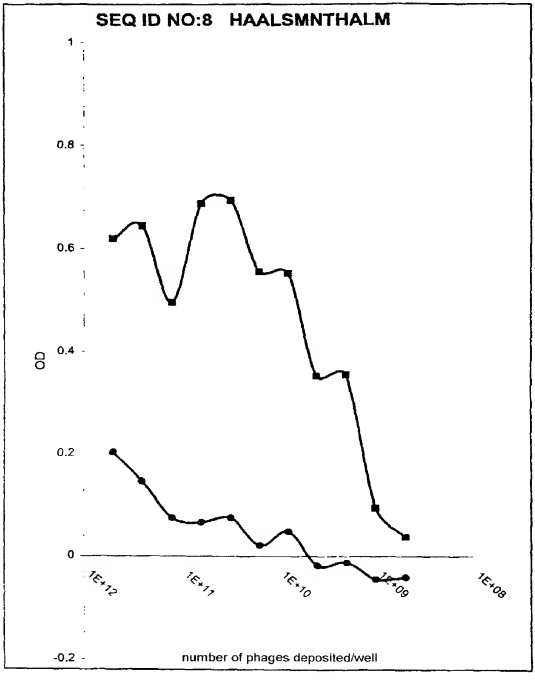
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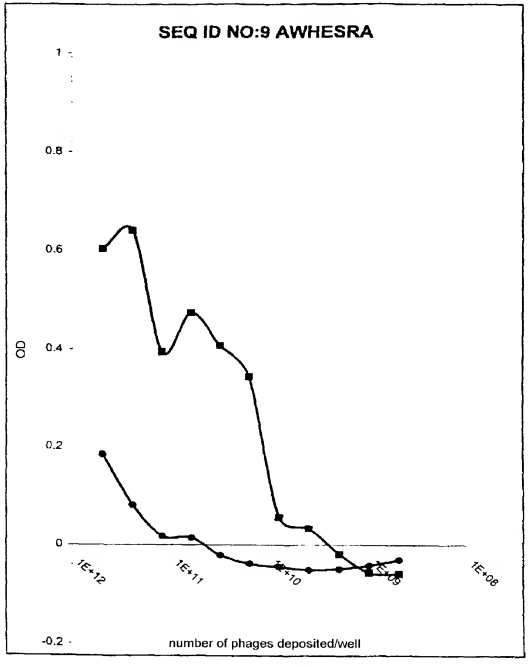
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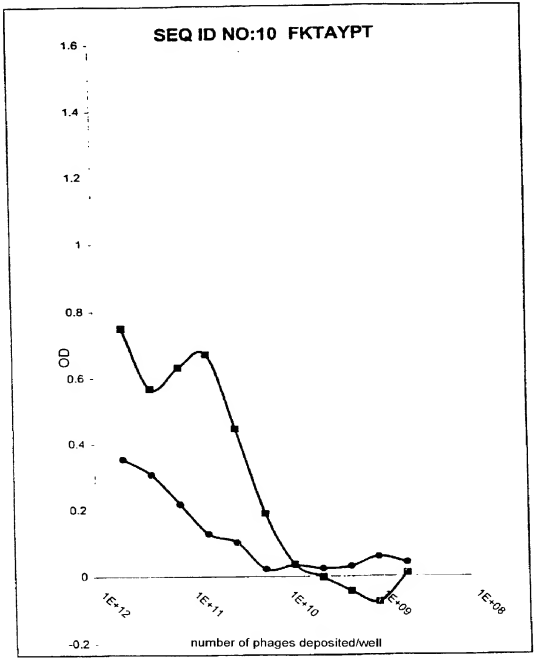
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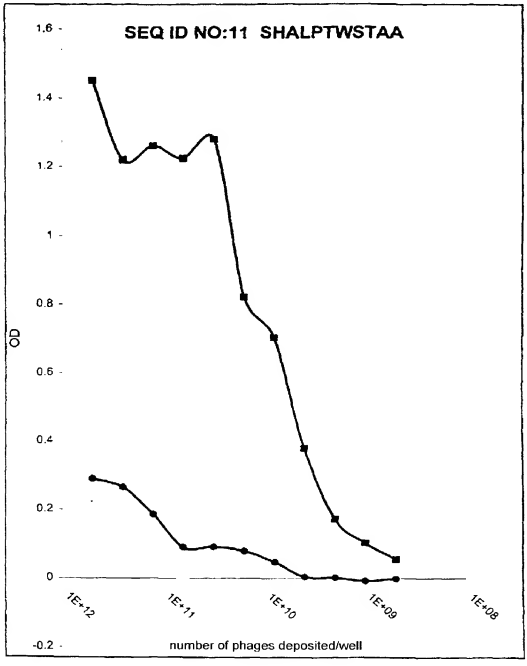
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DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

HIV Virus Mimotopes

the specification of which is attached hereto unless the following space is checked:

☒ was filed on **December 11, 2000** as United States Application Serial **09/719,437**.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

	Number	Country	Day/Month/Year Filed
1.	98/07598	FR	12/06/1998
2.			

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

	Application Number	Filing Date
1.		
2.		

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

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1.	PCT/FR99/01409	14 June 1999	pending
2.			

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I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and I direct that all correspondence be addressed to that Customer Number

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Principal attorney or agent: **Michael S. Greenfield**
Telephone number: 312-913-0001

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

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SEQUENCE LISTING

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